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SERIES ON INHALATION TOXICOLOGY

2. PERFORMANCE OF 69 LITRE INHALATION EXPOSURE CHAMBER AND ASSOCIATED SYSTEMS

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SERIES ON INHALATION TOXICOLOGY

2. PERFORMANCE OF 69 LITRE INHALATION EXPOSURE CHAMBER AND ASSOCIATED SYSTEMS

by
M.G. Prior and P.J. Henry
Alberta Environmental Centre

AUGUST 1992

DISCLAIMER

Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

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CONVERSION TABLE

To obtain	From	Multiply by
lumens m^2	foot candles	10.76
kPa	lbs sq. in.	6.895
mg m^{-3} hydrogen sulphide	ppm hydrogen sulphide	1.4
mg m^{-3} sulphur hexafluoride	ppm sulphur hexafluoride	6.15

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ABSTRACT

The Inhalation Toxicology Research Program of the Alberta Environmental Centre was developed in response to concerns expressed by government departments for the effects of toxic or potentially toxic gases, vapours and particulates on animals and, by extrapolation, man.

The testing of chemicals to determine their potential for toxicity following inhalation is the most technologically complex, difficult and expensive area of toxicology. It follows that careful consideration and evaluation of equipment and experimental design is essential.

A custom-designed building was constructed to provide the specialized facilities required. Several inhalation exposure systems are used for the study of the effects of acute exposure to toxic gases on laboratory animals. The theoretical requirements, design criteria, and components of inhalation exposure systems are described.

The performance characteristics of the inhalation system, based on a 69 litre exposure chamber, are described.

INTRODUCTION

Breathing is the one route of entry to the body that is least able to be controlled. It is possible to avoid ingesting noxious foods or feeds, to evade surface contamination by toxic chemicals, or to refrain from drinking contaminated water. It is not possible to avoid breathing, save for a few short minutes. Breathing is mandatory. A worker may inhale 30 million litres of workplace air during a working life; and a person may breathe some 250 million litres of air from cradle to grave (Clark, 1983).

Airborne Toxicants

The occurrence of airborne hazards in occupational situations, the presence of airborne pollutants in the environment, and the intensive use of agricultural chemicals, have focused attention on respiratory or inhalation toxicology. This discipline is associated with the requirement for special methods and equipment to study the effects of exposure of animal in experimental studies of airborne toxicants.

Testing of Pneumotoxicants

In order to respond to the concerns expressed about airborne pollutants by departments of the Government of Alberta, it was necessary to establish the specialized capability to undertake this work. The Inhalation Toxicology Research Program of the Alberta Environmental Centre was developed in response to the expressed need for information concerning the effects of toxic or potentially toxic gases, vapours and particulates on animals and, by extrapolation, man.

The testing of chemicals to determine their potential for toxicity following inhalation is the most technologically complex, difficult and expensive area of toxicology. It follows that careful consideration and evaluation of equipment and experimental design is essential.

Historical Perspective

Controlled animal inhalation exposure studies were described as early as 1865 by Eulenberg, who used a cubical wooden chamber with two glass walls for the exposure of small laboratory animals to high concentrations of numerous toxic and asphyxiant gases. Dust exposures were described by von Jus (1875), who studied the effects of diatomaceous earth. Lehmann and his associates, first at the Hygienic Institute in Munich and later at the Hygienic Institute of Wurzburg, published numerous articles on inhalation toxicology. The first paper, by

Lehmann (1886), describes an exposure apparatus for the exposure of cats, rabbits, guinea pigs and frogs. Lehmann and his associates published descriptions of inhalation chambers for gases (Dubitzki, 1911), for dusts (Saito, 1912), for mists (Lehmann *et al.*, 1912), and additional gas and vapour exposure units (Lehmann and Hasegawa, 1913). Silver (1940) described a 629 L chamber with a sliding cage carrier. The principles influencing the design and operation of constant-flow inhalation chambers for gas and vapour exposures were described by Silver (1946). Wilson and Laskin (1950) described a large hexagonal inhalation unit, which was later replicated in a smaller form by Leach and Spiegl (1956). MacFarland (1956) has described a chamber built for Health and Welfare Canada; this chamber was patterned after Silver's design (1940).

Objective of This Report

This report describes the design and evaluation of one inhalation exposure system, based on a 69 litre exposure chamber. This system is in use at the Alberta Environmental Centre for the study of the effects of inhalation exposure to toxic gases on laboratory animals. The components of this system are described, as well as their performance characteristics.

DESIGN CONSIDERATIONS FOR INHALATION SYSTEMS

Components of an Exposure System

A generalized dynamic inhalation exposure system consists of sources of air and toxicant (whether gas, vapour, particulate or aerosol), filters to purify and equipment to condition air temperature and humidity, flow controllers for air and toxicant(s), mixing devices, exposure chambers for animals, instrumentation for analysis of the chamber atmosphere, vacuum pumps, and detoxification of the exhaust from the exposure chamber. These components are depicted in diagrammatic form in Figure 1.

The characteristics of a dynamic inhalation exposure system, in which the airflow and introduction of agents are continuous, were originally described and verified by Silver (1946).

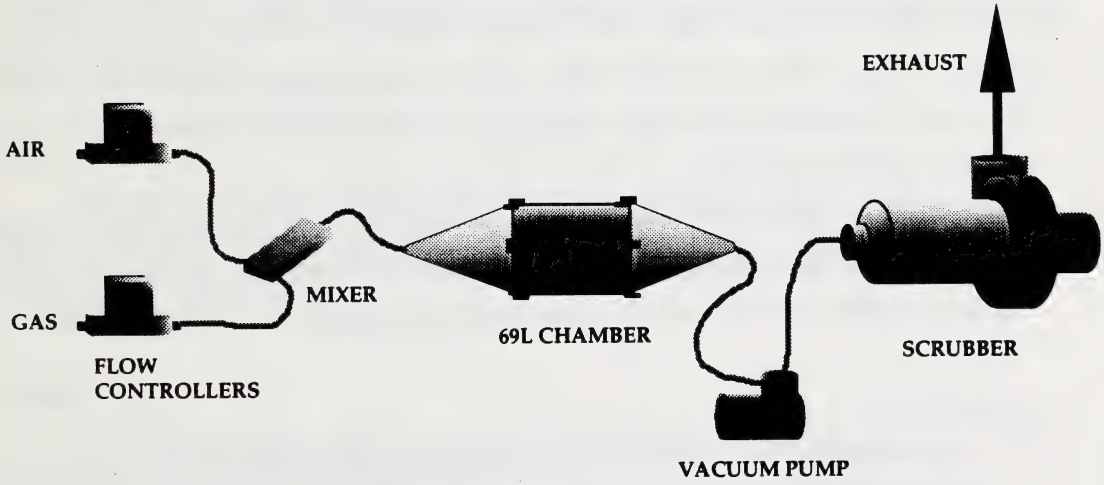

Figure 1. Schematic of generalized inhalation exposure system.

Air Flow

The supply air should be pre-conditioned with regulation of temperature and relative humidity, and removal of unwanted contaminants.

Chambers should be aerodynamically designed to ensure a uniformly distributed concentration of test material. They should be large enough for exposure of an adequate number of animals for statistical evaluation. They should be constructed of non-reactive materials which are easily cleaned. It should be possible to view the animals during exposure. An adequate number of ports for monitoring concentration, chamber pressure, temperature, and humidity should be included.

Inhalation systems are often operated at slightly negative atmospheric pressures relative to the laboratory to minimize the possibility of external contamination through leakage. The differential static pressure between the atmosphere within an inhalation chamber and the atmosphere in the laboratory should be monitored. Chamber effluent must be decontaminated before the release of exhaust gases into the environment.

Accurate regulation of airflow is essential and each component should be checked for performance. In most facilities exposure chambers are connected to common air supply and exhaust systems (Drew and Laskin, 1973). Airflows through the chambers vary from 10 to 60 air changes per hour. Since the amount of air required for heat removal is usually far in excess of that required to maintain oxygen and carbon dioxide balance, this amount constitutes the practical lower design limit for airflow.

Supply air should be passed through an absolute High-Efficiency-Particle-Absorber (HEPA), charcoal, and other appropriate types of filters before entry into the inhalation chamber. This is to ensure removal of noxious agents that might influence the experimental results.

Surface Effects

The interior surface of the chamber and auxiliary parts of the inhalation exposure system, to which the agent may be exposed, may lower its concentration because of absorption, adsorption, or chemical reaction. The magnitude of any surface effects depends on the quantity of surface exposed per unit volume of atmosphere. The surface area available for contact is controlled by the shape and size of the chamber and extraneous interior surfaces such as cages and air lines. The surface/volume ratio is largely a function of the size and shape of the chamber. A spherical chamber has the smallest surface/volume ratio. However, a cubical chamber is more easily constructed, and since it has a lower surface/volume ratio that the other rectangular shapes, it is the preferred form. Since the surface/volume ratio of either type of chamber is inversely proportional to the cube root of the volume, it decreases as chamber size increases (Silver, 1946). Losses to chamber surfaces can be reduced by the use of large volume chambers, non turbulent airflows, and electrically conductive interior surfaces. Non-metallic surfaces may acquire local regions of high charge that scavenge particles due to electrostatic forces (Phalen, 1984).

Spatial uniformity of airborne materials is often achieved by fitting the chamber with cone or pyramid-shaped entry and exit sections (Fraser *et al.*, 1959). In addition, uniformity is enhanced by proper injection and mixing of materials into the chamber air. Venturi sections and pre-mixing chambers may be necessary.

Sampling

Samples for characterization of exposure atmospheres should be taken from the breathing zone of the exposed animals, and be drawn through lines that do not compromise the sample. Sampling must be conducted during the actual exposure. Analysis is achieved by use of the most appropriate techniques.

Noise

The noise level should be held below 85 db to avoid stress of the experimental animals and human operators (Hobbs, 1982). Sources of noise and vibration include valves, motors, pumps and air movements.

Lighting

The recommended intensities for lighting are from 807 to 1345 lumens m^{-2}, although some authorities are suggesting 1130 lumens m^{-2} at 1.5 m above the floor level (Hobbs, 1982).

Animal Loading

Animal loading limits are based on the reactivity of animal surfaces with the exposure atmosphere, and the generation of heat and water vapour by the exposed animals. Heat generated in a stainless steel chamber is largely dissipated through the chamber walls (Bernstein and Drew, 1980). A total animal volume of 5% conforms to thermal requirements, and also to the loading limit proposed by Silver (1946) as the limit above which animal surface effects cause excessive concentration changes and may lead to unacceptable concentrations of ammonia.

Temperature

The temperature of the chamber should be in the range 22 ± 3°C (OECD, 1981; FIFRA, 1978), 23.3 ± 1.1°C (NIH, 1972) or 26°C (Hobbs, 1982), and should be recorded at least three times daily. Humidity should be in the range of 30 - 70% RH (OECD, 1981; FIFRA, 1978), or 40 - 60% RH (NIH, 1972). Generally, humidity is more difficult to control and measure accurately than temperature.

Stability of Exposure Atmosphere

The stability of the exposure atmosphere in a dynamic system involves keeping six factors constant:

1. Gas supply generator
2. Air flow
3. Pressure
4. Temperature
5. Humidity
6. Losses on interior surfaces

Fluctuating generation of gas or aerosol will change the measured concentration of the exposure atmosphere.

Air flow through a chamber influences:

1. the equilibration time to attain the theoretical constant concentration;
2. the concentration which can be established for any given agent.

Flow and pressure are inter-related. An airflow of 10 - 15 air changes per hour with the total animal bodyweight less than 5% of the total chamber volume should maintain an oxygen concentration of not less than 19%.

PERFORMANCE OF INHALATION SYSTEM WITH 69 L EXPOSURE CHAMBER

Exposure Chambers

The custom-designed and built exposure chambers (Figure 2) consist of a clear, acrylic right cylinder (constructed by Gary Jones Design Ltd., Edmonton, AB), with two removable stainless steel right cones (manufactured by J.K. Campbell Associates, Edmonton, AB). The custom-designed and built inhalation exposure chamber consists of a cylinder and two cones. In geometrical terms the cylinder is a RIGHT CYLINDER and the cones are RIGHT CONES. That is, the ends of the cylinder are at right angles to the longitudinal axis, and the apex of the cone lies on the axis drawn perpendicular to the geometric centre of the base of the cone.

The volume of a right cylinder is given by

$$V_{cyl} = \frac{N \ x \ R^2 \ x \ L}{10^3} \tag{1}$$

where
V_{cyl} = volume (L)
R = radius of right cylinder (cm)
H = height (length) of right cylinder (cm)

The surface area of a right cylinder is given by

$$S = 2 \ x \ N \ x \ R \ x \ H \tag{2}$$

where
S = surface area (cm^2)
R = radius of right cylinder (cm)
H = height of right cylinder (cm)

The volume of a right cone is given by

$$V_{cone} = \frac{N \ x \ R^2 \ x \ H}{3 \ x \ 10^3} \tag{3}$$

where V_{cone} = volume (L)
R = radius of right cone (cm)
H = height of right cone (cm)

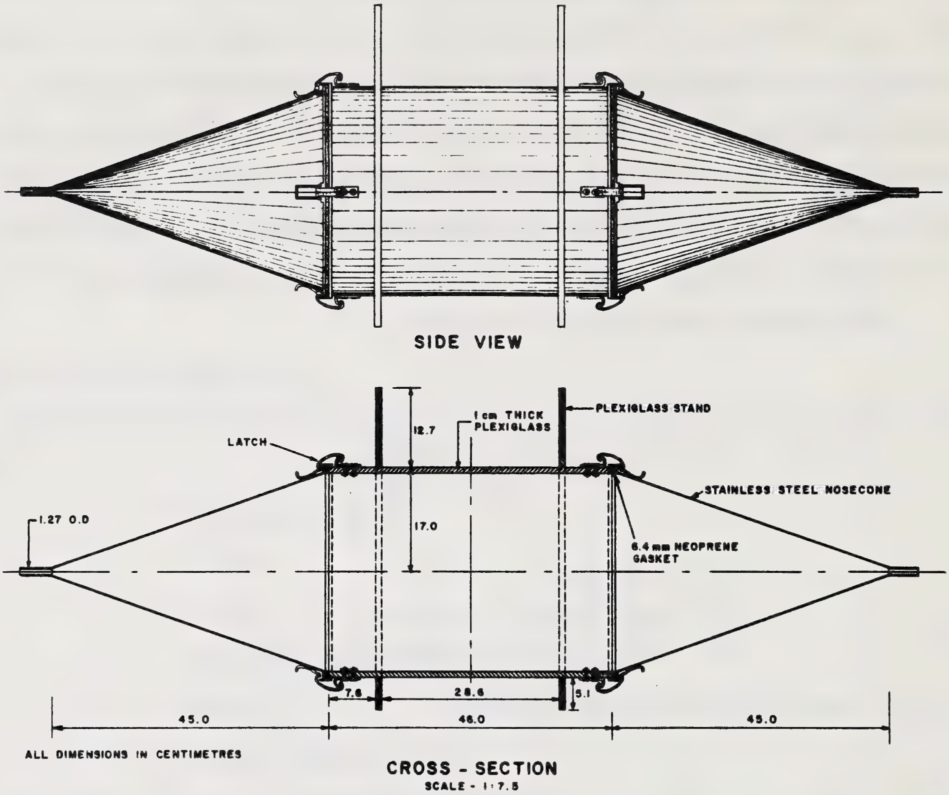

Figure 2. 69 L inhalation exposure chamber.

The surface area of a right cone is given by

$$S = N x R x S \tag{4}$$

and

$$S = (R^2 + H^2) - 2 \tag{5}$$

where S = surface area of right cone (cm^2)
R = radius of right cone (cm)
H = height of right cone (cm)

The volume of the novel inhalation chamber is given by

$$V_{chamber} = V_{cylinder} + V_{coneA} + V_{coneB} \tag{6}$$

where $V_{chamber}$ = volume of chamber (L)
V_{cyl} = volume of right cylinder (L)
V_{coneA} = volume of cone A (L)
V_{coneB} = volume of cone (L)

The ratio of volume to surface area of the inhalation exposure chamber is given by

$$Ratio = \frac{V_{cyl} + V_{coneA} + V_{coneB}}{S_{cyl} + S_{coneA} + S_{coneB}} \tag{7}$$

The dimensions of the chambers are given in Table 1. The chambers have a mean volume of 69.3 ± 0.2 L (Table 2). Each chamber holds three circular stainless steel mesh cages (manufactured by Alberta Public Works, Supplies and Services, Edmonton, AB), each holding 4 rats per cage in individual compartments.

Table 1. Measurements of 69 L exposure chamber.

Chamber		Cylinder		
		Radius (cm)	Length (cm)	Volume (L)
1	Cylinder	17.1	46	42.27
1	Cone A	16.85	45.9	13.65
1	Cone B	16.75	46.5	13.67
2	Cylinder	17.1	46.0	42.27
2	Cone A	16.8	46.4	13.72
2	Cone B	16.85	45.6	13.56
3	Cylinder	17.1	45.7	41.99
3	Cone A	16.8	46.0	13.6
3	Cone B	16.9	46.4	13.88
4	Cylinder	17.1	45.9	42.27
4	Cone A	16.8	45.8	13.54
4	Cone B	16.9	47.2	14.12

Table 2. Summary of measurements of 69 L exposure chamber.

Chamber Number	Volume (L)	Surface area (cm^2)	Ratio
1	69.59	10135.0	145.6
2	69.55	10124.8	145.6
3	69.47	10120.5	145.7
4	69.93	10171.8	145.5
Mean	69.6	10138.0	145.6
SD	0.2	23.3	0.1

Air Supply

Compressed air was cooled by passing through an air-to-air finned tube intercooler, and an air-to-air finned aftercooler to a storage tank. The air was then passed through a refrigerated compressed air dryer (Ingersall Rand Canada Inc., Edmonton, AB) to cool, dry and scrub it of hydrocarbons, and supplied at 551.6 x 10^3 Pa at the room.

Air connections between the components of the system were by 1/4 or 1/2 inch diameter teflon TFE (Chromatographic Specialities Ltd., Brockville, ON; Johnston Industrial Plastics Ltd., Edmonton, AB) tubing and Swagelok^{TM} fittings (Edmonton Valve & Fitting, Edmonton, AB).

Sources of Test Articles

Four substances were used for testing the chambers prior to experimental exposure of animals:

1 Sulphur hexafluoride, 99.8% C.P. (Matheson Gas Products, Edmonton, AB);
2 Acetone (Fisher Scientific, Edmonton, AB);
3 Obscurant smoke (Superior Signal Company, Spotswood, NJ);
4 Hydrogen sulphide, 99.5% C.P. (Matheson Gas Products).

Sulphur hexafluoride and hydrogen sulphide were contained in gas cylinders, passed first through a two-stage regulator and maintained at a pressure of 413.7 x 10^3 Pa. Liquid acetone was injected directly into the air supply line. Obscurant smoke was generated from a smoke candle.

Test Article Generation

The stability of generated atmospheres within the exposure chamber is important, both from consistency of the maintained concentration and the aspect of distribution of the test article within the exposure chamber. Sulphur hexafluoride and hydrogen sulphide were supplied from gas cylinders under pressure, and were disconnected when the cylinder pressure dropped below 3.447 x 103 Pa in order to maintain constant pressure and flow.

Mixing Devices

The two gases (sulphur hexafluoride or hydrogen sulphide) were dynamically mixed by opposing stream mixing, and then passed through a turbulator (Tah Industries Ltd., NJ). Aliquots of acetone were injected directly into the supply air line, approximately 10 cm from the apex of the supply-side cone. Obscurant smoke was introduced by placing the ignited smoke candle into a spool section of the supply air line, and the distribution of obscurant smoke within the exposure chamber was recorded by still camera.

Flow Control

Accurate control and measurement of air flow is essential. Needle valves, rotameters, mass flow controllers and vacuum pump were tested for accuracy and precision. A dry gas meter (Canadian Meter, Division of Singer Company of Canada Ltd., Edmonton, AB) was used for measurement of air flows for the mass flow controllers and higher capacity rotameters (greater than 1 L min^{-1}). This meter was first calibrated against a wet air meter (GCA/Precision, Chicago, IL). The correction factor (wet/dry) was 0.97. A soap bubble meter (Hewlett Packard Ltd., Edmonton, AB) was used to measure air flow through the needle valves and lower capacity (less than 1 L min^{-1}) rotameters. Time was recorded using a digital stopwatch (Canlab Ltd., Edmonton, AB).

Two types of flow controllers were used: rotameters (Dwyer Instruments Inc., Michigan City, MI; Matheson Gas Products), and mass flow controllers (Matheson Gas Products). The results of air flow measurements for the needle valves and rotameters are summarized in Table 3. The flows of test article and air were adjusted to maintain the target concentration in the test chambers, and these values used to calculate the nominal concentration.

Air flow rates and pressure are inter-related. Supply air is pushed through the AEC inhalation system by a compressor pump, and in addition, a downstream air vacuum pump draws air through the system. In general the air flows were relatively constant, there being less than 2% variation save for the needle valves (Table 3). Flow through the system is therefore stable provided that no significant change in the resistance to flow occurs.

Table 3. Summary of flow measurements for various flow control devices.

Device	Airflow (Ml min^{-1}) (mean ± Std Dev)	Coeff. of Variation
Needle Valve	1.7 ± 0.1	5.9
	2.5 ± 0.1	4.0
	4.3 ± 0.1	2.3
	9.5 ± 0.4	4.2
Rotameter	16,200 ± 200	1.2
	16,600 ± 200	1.2
	1,240 ± 20	1.6
	324,620 ± 1,980	0.6
Mass flow controller	16,600 ± 200	1.2
	16,500 ± 200	1.2
Vacuum pump	39,900 ± 300	0.8

Chamber Monitoring Systems

The test chambers were maintained at a (negative) pressure of -74.6 Pa for the duration of the tests. A gas chromatographic monitoring system was employed to analyze air samples obtained by automatic sampling methods. Automatic chamber monitoring was achieved using a pneumatically operated sampling system (Valco Instruments, Houston, TX). This system operated using a continuous flow through a 14 port multiple stream complex (Valco Instruments) (Figure 3) in conjunction with a gas sampling valve (Valco Instruments) (Figure 4) and metal bellows vacuum pump (Model MB-2, Metal Bellows Corp., Sharon, MA). The 14-port valve was connected to the gas chromatograph via the carrier inlet and analytical column. A calibrated sample loop was used to control the injection volume. A digital valve controller determined the frequency of injections and started the analogue to digital conversion.

Each sample line was sampled approximately two times per hour. For determination of operating characteristics each chamber was fitted with either ten (sulphur hexafluoride, hydrogen sulphide) or two (acetone) sample lines. For exposure of animals each chamber was fitted with

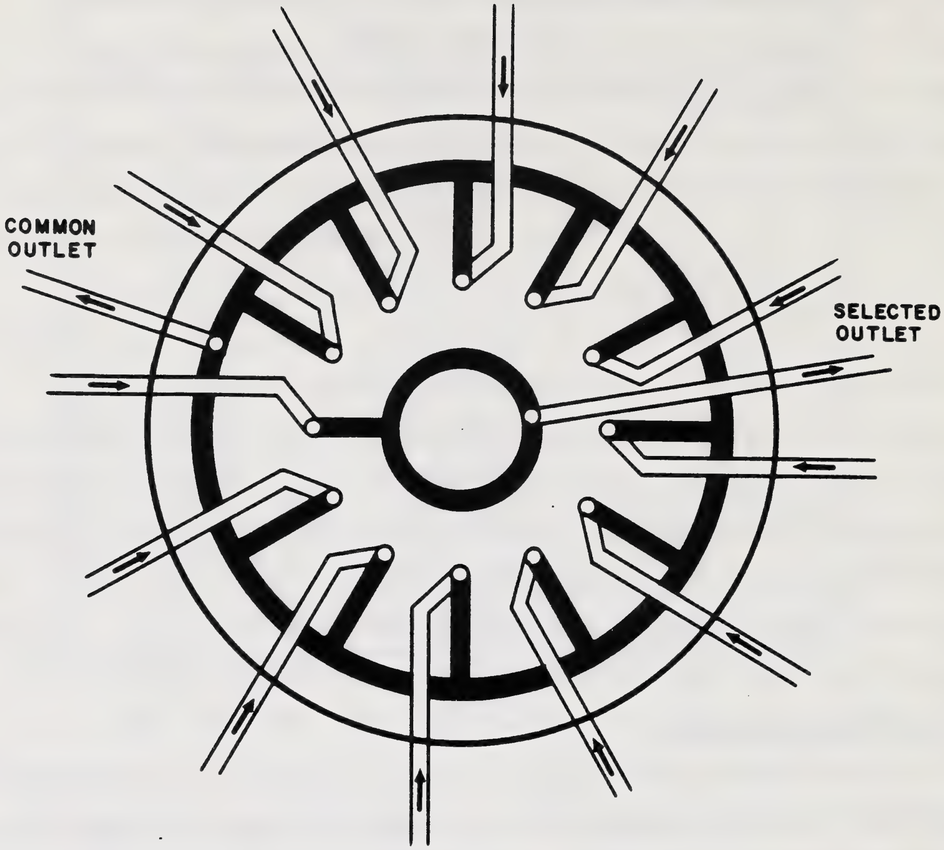

Figure 3. 14-port multiple stream sampling valve.

three sample lines, one in the supply line and two in the chamber. Control atmospheres were also sampled approximately four times per hour. Exposure room air was monitored continuously for hydrogen sulphide using a Hydrogen Sulphide Monitor (Model R-17, Interscan Corp., Chatsworth, CA). The gas chromatograph and monitoring system instruments were located in a room adjacent to the exposure chambers; there was no exchange of room air between the two. The samples were analyzed for sulphur hexafluoride or hydrogen sulphide using a Gas Chromatograph (Model 5790A, Hewlett Packard), with a Sample/Event Controller Module (Model 19400A, Hewlett Packard), and an Integrated (Model 3390A, Hewlett Packard). Analysis was achieved using a 2 x 0.003 m, 80/100 mesh, silanized porous ethylvinylbenzene-divinylbenzene copolymer bead column (Poropak QS™, Chromatographic Specialities, Brockville, ON) and Flame Photometric Detector (Hewlett Packard).

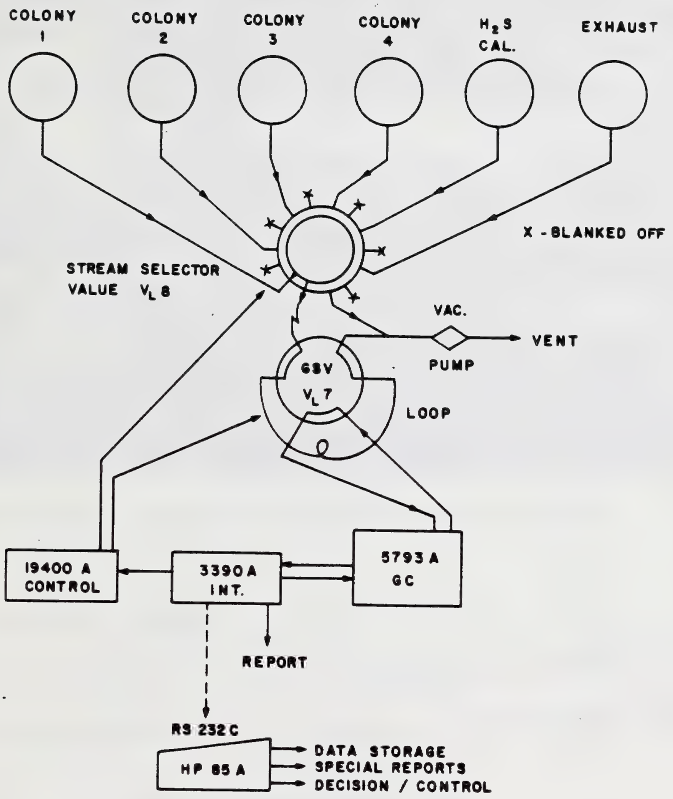

Figure 4. Distribution of obscurant smoke within 69 L exposure chamber.

Acetone was used as the tracer for the determination of dead space and turbulence. Aliquots of acetone were injected into the supply air line approximately 10 cm before the inlet of the 69 L exposure chamber. A sample line was placed either at the inlet or the outlet of the chamber. The sample was drawn through the line using a vacuum pump (Model MB-2, Metal Bellows Corp., Sharon, MA) to a gas chromatograph (Model GC 301, HNU). The carrier gas was air, and the sample detected by Flame Ionization Detector. A blank 45.7 cm copper tube served as the column. Oven temperature was 50°C; detector temperature 150°C. Peak height, shape and time were recorded on a chart recorder (Model LM23, Linseis). The runs were repeated at least three times. A typical chamber response curve for acetone is shown in Figure 5.

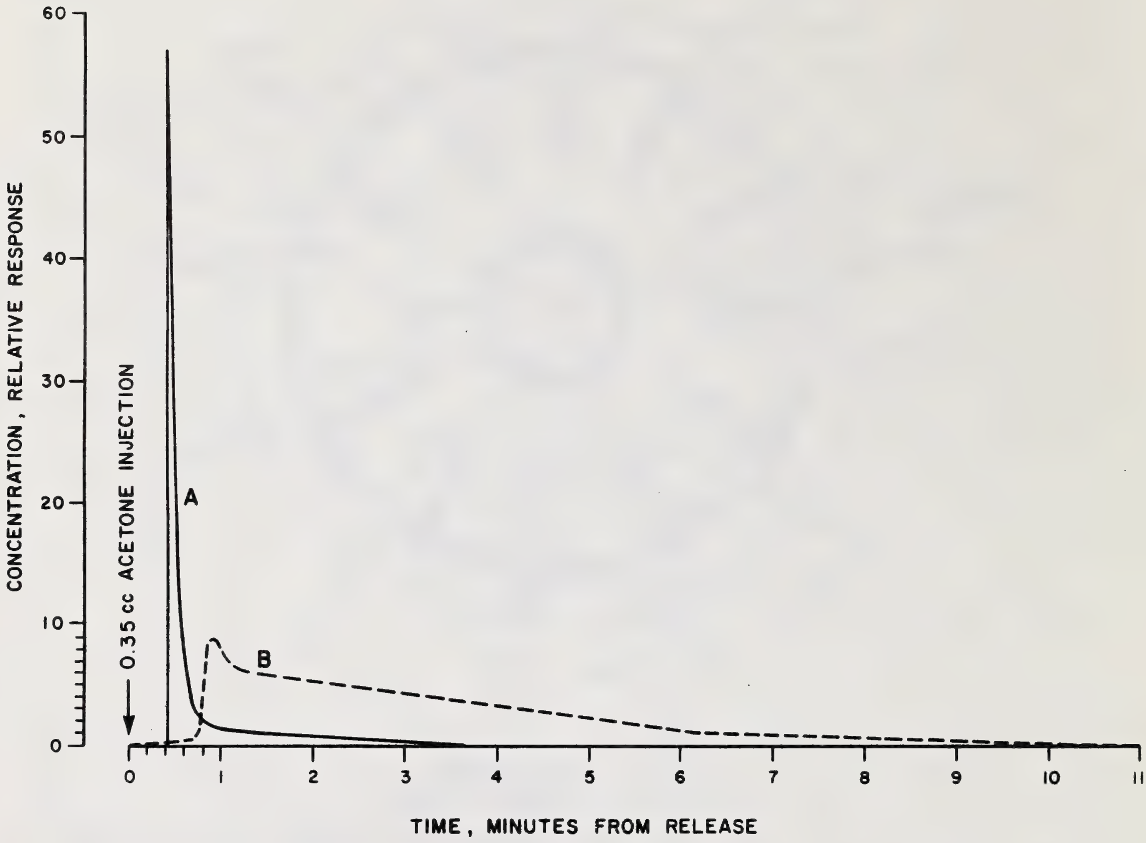

Figure 5. Gas sampling valve.

A smoke candle (Superior Signal Company) was placed within the supply air line and ignited. A film record was obtained using colour print film (Kodacolour™), single lens reflex camera with electronic flash (Pentax Canada Inc., Edmonton, AB). A digital stopwatch (Canlab Ltd., Edmonton, AB) was placed in the field of view to provide timing of the sequence.

A typical view is presented in Figure 6. These indicate that the smoke was distributed throughout the chamber within two minutes of commencement.

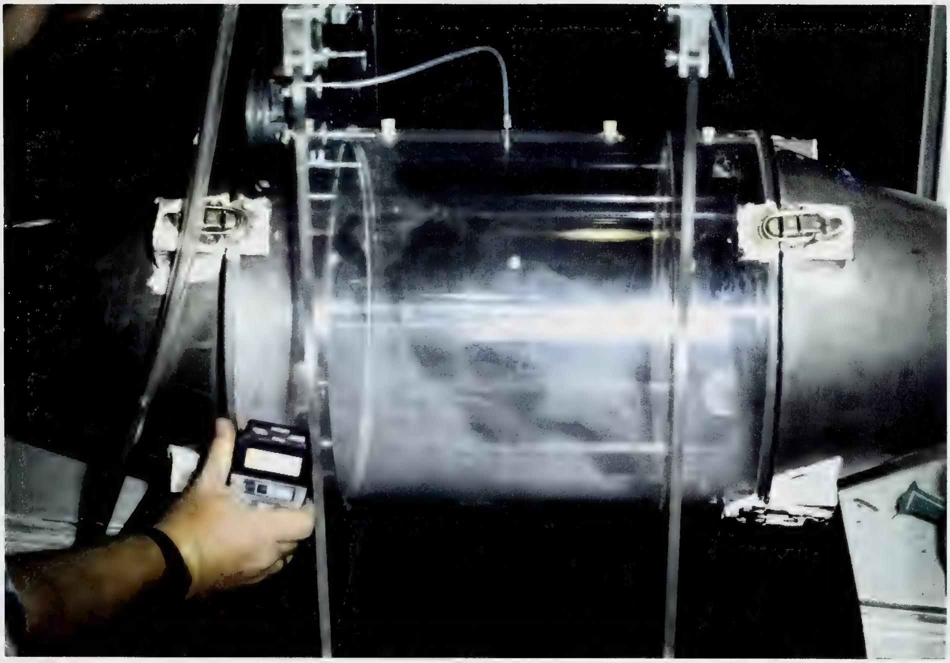

Figure 6. Distribution of obscurant smoke within 69 L exposure chamber.

Gas Distribution Within 69 L Chamber

The nominal concentration of an agent in a chamber can be calculated from:

$$CN = \frac{FC}{FA} \tag{8}$$

where CN = nominal concentration of chemical
FC = flow of chemical
FA = flow of air

Additional factors contributing to differences between the nominal and actual concentrations obtained in a chamber include animal uptake, absorption onto animal fur, and variations in flow.

Upon introducing an agent into the airflow, the concentration in the chamber increases until it becomes constant, or reaches an equilibrium. Assuming perfect mixing:

$$C = \frac{w}{b}\,[1 - \exp^{bt/a}] \qquad (9)$$

where C = concentration of agent at time t
w = amount of agent introduced per minute
a = volume of chamber
b = flow of air through chamber

From these equations it is possible to calculate the theoretical variation in toxic gas atmosphere concentration due to variation in air flow. These are summarized in Table 4. These theoretical calculations show a coefficient of variance of less than 3%, when the measured extremes of air and gas flows are plotted. A 5% variation in the concentration of the chamber atmosphere is generally regarded as acceptable.

Concentrations of 150, 300, 600 or 1200 mg m^{-3} sulphur hexafluoride in nitrogen (Matheson Gas Products) were supplied without dilution to the exposure chamber at a flow rate of 16 L min^{-1}. The chamber was sampled on a grid pattern and the results are summarized in Table 5. After equilibration, the coefficient of variation was equal to or less than 5% in all cases. This is generally regarded as acceptable.

In addition to verifying the distribution of the test article within the inhalation chamber, it is also necessary to ensure that the dynamic mixing of test article and carrier gas (supply air) is acceptable. The results of dynamically mixing a single source concentration of sulphur hexafluoride with air to achieve five different chamber atmosphere concentrations is summarized in Table 5. The distribution of nominal concentrations of 156, 462, 480, 1079 or 1211 mg m^{-3} sulphur hexafluoride obtained by dynamic mixing are given in Table 6. Ideally, the Actual/Nominal (A/N) ratio should be unity. It is noted that the A/N ratio is within 1% of unity, and that the Coefficient of Variation is within 5%. This is regarded as acceptable performance.

Table 4. Theoretical variations in chamber atmosphere concentrations due to variations in air and gas flow.

Air Flow (L min^{-1})	Gas Flow (Ml min^{-1})	Starting Concentration (mg m^{-3})	
		1393000	431
16.8	1.26	104.4	
16.3	1.26	107.6	
16.8	1.22	101.2	
16.3	1.22	104.3	
16.6	1.22	102.3	
Mean ± Std. Deviation		103.9 ± 2.5	
Coefficient of Variation		0.02	
16.8	4.54	376.4	
16.3	4.54	387.9	
16.8	4.44	368.2	
16.3	4.44	379.4	
16.6	4.49	376.7	
Mean ± Std. Deviation		377.7 ± 7.1	
Coefficient of Variation		0.2	
16.8	326.6		8.4
16.3	326.6		8.6
16.8	322.6		8.2
16.3	322.6		8.5
16.6	324.6		8.4
Mean ± Std. Deviation			8.5 ± 0.2
Coefficient of Variation			0.02

Table 5. Variation within 69 L chamber of calibration (non-diluted) concentrations of sulphur hexafluoride at flow of 16 L min^{-1}.

Concentration (mg m^{-3})			A/N Ratio	Coefficient of Variation	Concentration (mg m^{-3})	
Nominal	Actual					
	Mean	Std Dev			Maximum	Minimum
150	149.9	12.6	0.999	0.09	176.3	129.5
150	155.9	7.2	1.04	0.05	168.5	143.9
300	303.3	7.2	1.012	0.02	321.9	292.6
600	603.7	20.4	1.007	0.03	636.7	574.3
1200	1198.4	17.4	0.999	0.01	1226	1172.6
1200	1199.6	41.4	0.999	0.03	1239.2	1080.9
1200	1195.5	21	0.997	0.02	1224.2	1155.8

Table 6. Variations in chamber concentrations with dynamic mixing of sulphur hexafluoride and air at combined flow of 16 L min^{-1}.

Concentration (mg m^{-3})			A/N Ratio	Coefficient of Variation	Concentration (mg m^{-3})	
Nominal	Actual					
	Mean	Std Dev			Maximum	Minimum
156	156	7	1.000	0.04	169	144
462	462	6	1.000	0.01	489	450
480	480	10	1.000	0.02	489	466
1079	1080	16	1.001	0.03	1113	1016
1079	1078	14	0.999	0.01	1096	1045
1211	1211	20	1.000	0.04	1252	1092

Exhaust System

Exhaust air from the test chambers was drawn first through a 1% solution of sodium hydroxide (Fisher Scientific, Edmonton, AB), by vacuum pump (Model 7MD-HC, Little Giant Pump Co., Oklahoma City, OK) with electric motor (Baldor Electric Co., Fort Smith, AR); then via a blower (maximum capacity 9 167.5 L min^{-1} through a portable fume scrubber (Model HS-7, Mystaire, Plainview, NY) containing an 6% sodium hypochlorite solution, before being released into the atmosphere via the inhalation exposure hood exhaust.

Temperature and Relative Humidity

A combined probe (Model 5124, Weathertronics, Sacramento, CA) was used to measure temperature (thermistor) and relative humidity (thin fibre capacitive sensor). This was connected to a Signal Conditioning Module (Model 1005, Weathertronics), Power Supply Module (Model 1030, Weathertronics), Temperature Module Thermistor Input (Model 1419, Weathertronics), RH Module (Model 1500, Weathertronics) with Digital Display (Model 1990, Weathertronics). In addition, the temperature and relative humidity were plotted on a two channel X-recorder (Model D5217, Houston Instruments, Austin, TX).

After acclimatization, the chambers were loaded with 12 rats, and the relative humidity and temperature recorded. The Time-Weighted Average (TWA) are given in Table 7. In agreement with Silver (1946), the relative humidity was found to be the limiting factor. These results indicate a maximum loading of 4 500 g total bodyweight.

Table 7. Relative humidity and temperature data.

Total Bodyweight of rats (g)	Relative Humidity (%) TWA[1]	Temperature (°C) TWA[1]
1932	34 ± 4	23.1 ± 0.3
2703	48 ± 14	23.3 ± 0.2
3509	48 ± 10	23.4 ± 0.1

Temperature and relative humidity require conditioning of the supply air. In the present inhalation system, temperature varied only slightly, and was virtually constant (Table 7). Relative humidity was more variable, and is a function of water vapour content and air temperature. Metal surfaces, such as the right cones of the inhalation chambers, conduct heat rapidly and make it more difficult to operate chambers at temperatures that differ greatly from the surrounding air temperature. Relative humidity is also dependant upon the animal mass within the exposure chamber and the air flow through the inhalation chamber. The performance of these two parameters is within the recommended ranges (OECD, 1981; FIFRA, 1978; NIH, 1972).

Health and Safety

Health and safety guidelines have been prepared for the operation of the inhalation exposure system, in conjunction with AEC staff, and provincial government departmental safety officials (Procedures Manual, 1990).

QUANTITATIVE CHAMBER PERFORMANCE

Gas Mixing

The most widely used method of mixing two or more gases is to dilute the gases with one another after measuring their flow rates. They are combined to a single test mixture after passing through a mixing chamber.

The concentration calculation for a single dilution dynamic system is:

$$C_a = \frac{10^2 \times q_a}{q_a + q_b + \dots q_n} \qquad \textbf{(10)}$$

where
C_a = concentration of component *a*
q_a = flow rate of component *a*
q_b = flow rate of component *b*
q_n = flow rate of component *n*

If component *a* is not pure, this equation becomes:

$$C_a = \frac{10^2 \times Xq_a}{q_a + q_b + \dots qn} \qquad \textbf{(11)}$$

where X = mole fraction, or decimal percent, of purity by volume.

The concentration in parts per million can be calculated from:

$$C_a + \frac{10^6 \times q_a}{qD} \qquad \textbf{(12)}$$

where qD = diluent gas flow rate

If the component gas is not pure, then:

$$C_a + \frac{10^6 \times Xq_a}{qD} \qquad \textbf{(13)}$$

Equilibrium

It is desirable to know how long it takes for a given gas stream to fill a chamber of known dimensions. Assuming perfect and instantaneous mixing, re-arranging Equation 13, and taking logarithms:

$$2.303\log\frac{C_0}{C_0-C} = \frac{bt}{a} \tag{14}$$

where C_o = initial concentration

Therefore:

$$t = 2.303\,\frac{a}{b}\log\frac{C_0}{C_0-C} \tag{15}$$

or:

$$t_x = K\frac{a}{b} \tag{16}$$

where x = percent nominal concentration attained in time *t*
K = constant

Values for K for various values of x can be calculated and are listed in Table 8. Silver (1946) verified these values of K using a number of compounds and chambers.

Table 8. Constants reported by Silver (1946).

x	K
99.9	6.9
99	4.6
95	3
90	2.3
85	1.9
80	1.6

Distribution Parameters

Conventional chambers may demonstrate poorly distributed flow patterns, with uneven distribution of chamber concentrations (Carpenter and Beethe, 1980). In practice it is difficult to define the active mixing volume within an exposure chamber on a quantitative basis. The use of smoke tubes and other visualization techniques can provide visualization of chamber behaviour but provides non-quantifiable information. In general, a model of the overall behaviour of a material can be derived from a mass balance of the system incorporating a dispersion coefficient of the degree of turbulence and hence the rate of dispersion. The percent dead space can be calculated from the measured mean residence time in comparison to the theoretical value obtained from chamber dimensions and flowrate.

A model of the overall behaviour of a material can be derived from a mass balance of the system incorporating a dispersion coefficient as representative of the degree of turbulence and hence the rate of dispersion. To simplify matters, the dispersion will only be considered in one dimension and, hence, will represent transport in a tube, ignoring any radial dispersion.

The mass balance of an element within the chamber will be found to be given by

$$U_z \frac{d_{C_i}}{d_z} = DL \frac{d^2 C_i}{d_z^2} + R_i \tag{17}$$

where U_z = average velocity transport into the element
z = direction along axis of transport
DL = axial dispersion coefficient
C_i = concentration of the *i*th component
R_i = rate of reaction of the *i*th component

In passing, it is noted that the ideal tracer should have the following properties:

1 minimal volume displacement of the chamber flow;
2 easily detected over a range of concentrations;
3 creates no change in the physical properties of the airstream;
4 not be adsorbed or undergo a chemical reaction.

Theoretical Mean Residence Time

The theoretical mean residence time of a chamber is defined as:

$$R_{theo} = \frac{V}{F} \tag{18}$$

where V = volume of chamber
F = flowrate through chamber

Assuming no loss of flow due to reaction, the measured residence time may be defined as

$$R_{meas} = \frac{VA}{F} \tag{19}$$

where VA = the active volume through which the airstream is moving.

The calculated theoretical mean residence time was 4.19 ± 0.02 min.

The exponential model is based on the assumption that an exposure chamber behaves as a completely mixed vessel (Drew and Laskin, 1973). For ideal chamber performance, if the system were to have no dead space and have true laminar flow (DL = 0; $R_{meas} = R_{theo}$) then the chamber outlet response would be instantly up to the chamber equilibrium value; when the system was shut off it would drop instantly to zero after one residence time. If instead the chamber flow patterns are completely mixed (high turbulence, DL = ∞) and there is no dead space ($R_{meas} = R_{theo}$), then the chamber would exhibit an exponential increase and decrease in concentration on stairhead and shut-down. Under these circumstances it would take approximately 4.605 residence times to achieve a concentration that was 99% of the equilibrium value. These two theoretical possibilities are depicted in Figure 7.

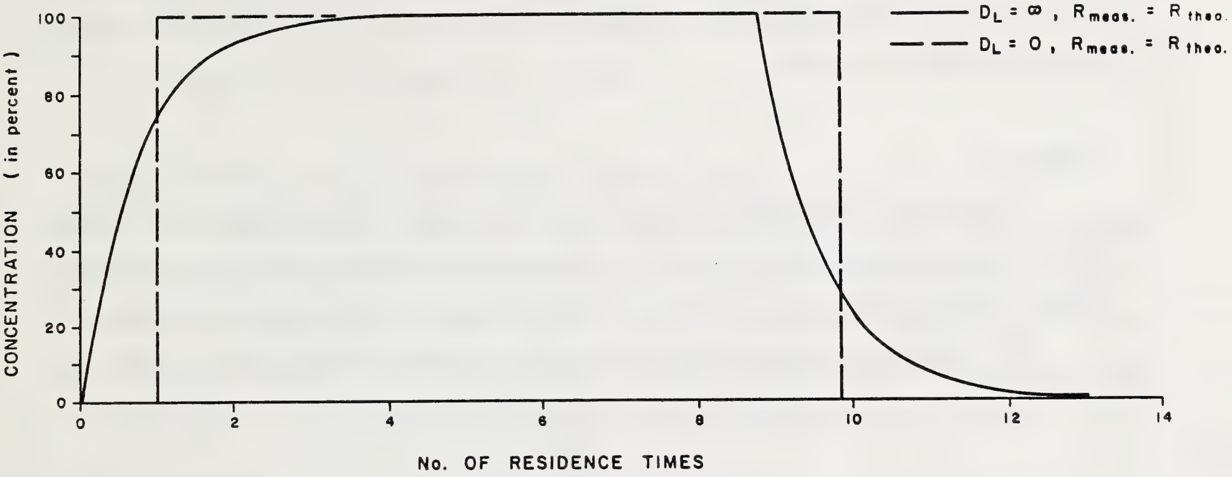

Figure 7. Theoretical response concentration curves dependent upon chamber design.

Observed Mean Residence Time

In practice it is difficult to quantify the active volume within a chamber. The mean residence time can be measured in an exposure chamber by calculating the time of passage of an impulse input of tracer material. The measurement of the tracer passage requires independent measurement at two distinct points as well as measuring the concentration as a function of time.

The mean residence time can be calculated from

$$\bar{t} = \frac{\sum_{i=0}^{n} C_i \, t_i}{\sum_{i=0}^{n} C_i} \tag{20}$$

By knowing the mean time of passage to two distinct points of the same tracer cloud, the measured mean residence time can be calculated from

$$R_{meas} = t_2 - t_1 \tag{21}$$

assuming point 2 is at or near the outlet of the chamber and point 1 is at or near the inlet. For the 69 L chamber the mean observed residence time was 0.52 ± 0.1 min for the inlet and 3.37 ± 0.24 min for the outlet.

Turbulence

The measure of the degree of turbulence of the system, the axial dispersion coefficient (DL) can then be calculated by knowing the increase in the variance of the concentration-time profile of the tracer from point 1 to point 2 (Himmelblau & Bischoff, 1968; Rich, 1973).

The dispersion or variance of the tracer profile is calculated for equal time intervals by

$$a^2 = \frac{\sum_{i=0}^{t_i - t_1} C_i}{\sum_{n}^{C_i}} \tag{22}$$

Therefore the axial dispersion coefficient is given by

$$DL = \frac{L^2 \, (a_2 t_2 - a_1 t_1)}{2 \, R_{meas}^3} \tag{23}$$

where L = distance between the two sample points.

For the 69 L chamber the calculated mean axial dispersion coefficient was 0.15 ± 0.04 $m^3 sec^{-1}$.

Dead Space

The chamber dead space may be defined as

$$\% \ Dead \ Space = \frac{R_{theo} - R_{meas}}{R_{theo}} \times 100 \qquad (24)$$

where R = mean residence time of the tracer in the chamber.

The calculated dead space was 32 ± 6.6%.

Losses on Walls, Animals, Animal Cages and Other Surfaces

The materials used in construction of the inhalation system and which would be in contact with the toxic atmospheres were chosen for their non-reactivity and non-absorption. However, no material is totally inert. In addition, some of the toxic material will be absorbed onto the fur of the exposed animals. From the theoretical and observed measurements of gas atmospheres, it appears that the total losses to walls, animals, and other surfaces is less than 3%.

Quantitative Chamber Performance

The theoretical and observed residence times and dead space were calculated (Hemenway *et al.*, 1979), and are summarized in Table 9. The variance for inlet and outlet, and axial dispersion coefficients were calculated (Hemenway *et al.*, 1979), and are summarized in Table 10. These values are comparable to other horizontal flow chambers.

The theoretical performance of the 69 L exposure chamber can be calculated from the exponential equation to plot the expected increase in chamber atmosphere concentration when introducing the vapour. This theoretical curve has been plotted against the vapour concentrations actually detected in Figure 8.

Table 9. Variance for inlet, outlet, measured residence times and axial dispersion coefficients.

Chamber Number	Distance between sample points (m)	Variance t_i (min)	Variance t_o (min)	Axial Dispersion Coefficient (m^2sec^{-2})
1	1.415	0.11	3.85	0.12
2	1.415	0.98	3.18	0.15
3	1.454	0.64	3.65	0.17
Mean	1.428	0.28	3.56	0.15
SD	0.023	0.26	0.28	0.04

Table 10. Measured and theoretical residence times and dead space.

Chamber Number	Volume (L)	Airflow ($L\ min^{-1}$)	Theoretical Residence Time (min)	Mean Residence Time (min)		Measured Residence Time (min)	Dead Space (%)
				Inlet	Outlet		
1	69.59	16.53	4.21	0.47	3.65	3.18	24.5
2	69.55	16.53	4.19	0.46	3.19	2.73	34.8
3	69.47	16.53	4.18	0.64	3.28	2.64	36.8
Mean	69.54	16.53	4.19	0.52	3.37	2.95	32
SD	0.06	0	0.02	0.1	0.24	0.46	6.6

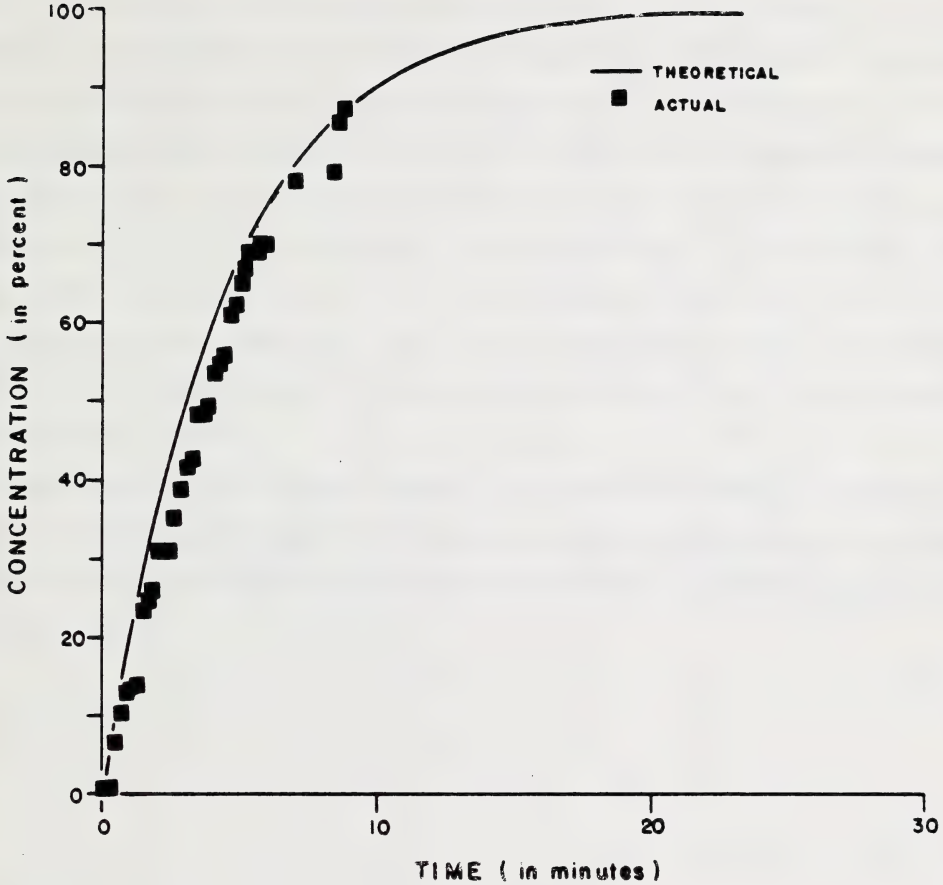

Figure 8. Theoretical versus actual concentration of acetone in 69 L exposure chamber.

CONCLUSIONS

The AEC inhalation exposure system, based on the 69 L exposure chambers, is capable of providing reproducible atmospheres for the study of toxic gases. The novel 69 L exposure chamber performs as well as other horizontal flow exposure chambers (Hemenway *et al.*, 1979).

The mean theoretical residence time was 4.19 ± 0.02 min; the mean measured residence time was 2.95 ± 0.46 min; the axial dispersion coefficient was 0.15 ± 0.04 m^2sec^{-1}; and the mean dead space was 32.0 ± 6.6%. The implication of these calculations is that the chambers will equilibrate within a reasonable period of time, with satisfactory mixing of air and toxic gas. These conclusions are confirmed by the measurement of distribution of sulphur hexafluoride within the chamber, whether by use of a known concentration of gas (Table 5) or by dynamic mixing of gas and air (Table 6).

The performance of the inhalation exposure system with 69 L horizontal flow chambers is suitable for the acute single exposure or short-term, multiple exposure types of inhalation studies. It would not be suitable for the longer term, multiple studies, or for those requiring large number of test animals.

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